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Morphological response of planktic foraminifers to habitat modifications associated with the  
emergence of the Isthmus of Panama

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## Abstract

The impact of global change on marine ecosystems is a major concern for the future. Examples from the geological past may provide insight into how ecosystems respond to major shifts in environment. Here we use the progressive closure of the Central American Seaway over the last 10 Myrs, and the resulting new environmental conditions and niches on either side of the Panama Isthmus, as a time series documenting the reaction of planktic foraminifers to environmental change and vicariance. Our main finding is that the size and shape evolution of both investigated species is strongly influenced by temperature, despite their different ecology. The surface dweller *Trilobatus sacculifer* conserved the same shape on both sides of the Isthmus for most of the studied interval, and diverged only recently when environment diverged on both sides of the Isthmus. The shape response is a combination of a change in mean shape and in percentage of morphotypes occurring within *T. sacculifer*. This suggests a minor role of vicariance and the potential to react to changes in the local environment through ecotypic or plastic variation. This interpretation is corroborated by extensive phenotypic variability in the absence of genetic differentiation today in this species. The shape of the deeper-living species *Gt. tumida*, in contrast, diverged on both sides of the Isthmus at a time that coincides with the cut-off of the connection of its habitat. This divergence combines a response to temperature and to location, suggesting local adaptation in response to vicariance. These different reactions highlight both a high potential for adaptation, but also sensitivity to temperature variations. The species-specific responses to environmental pressures indicate the difficulty in upscaling from one species to foraminifers in general.

37 Highlights:

38 The reaction of *T. sacculifer* and the *Gt. plesiotumida* – *tumida* lineage to the formation of the  
39 Panama Isthmus is assessed.

40 Temperature is the major driver of size changes.

41 Temperature is the major driver of the shape response of the surface dweller whereas  
42 vicariance additionally influences the deep dweller.

43 Species-specific responses to environmental pressures highlight the complexity of upscaling  
44 from species to the entire group.

45 Keywords: Planktic foraminifers, climate change, morphometrics, Isthmus of Panama

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## 1. Introduction

The impacts of current global change have become a major issue in research fields as diverse as palaeoceanography, ecology, evolution and epidemiology (Dam, 2013; IPCC, 2014; Sanford and Kelly, 2011). Environmental conditions within this century are predicted to be outside of the ranges experienced by a large number of species in their evolutionary history (Pörtner et al., 2014; Ridgwell and Schmidt, 2010) posing a significant threat. The majority of studies have focused on the effects on terrestrial ecosystems (Austin and Rehfish, 2005; Scholes et al., 2014); identifying coherent responses to global change in the marine environment has proven to be challenging (Poloczanska et al., 2013; Reusch, 2014). Marine plankton can be affected by drivers such as warming either directly via metabolic rates or indirectly via increases in surface water stratification, limiting nutrient input (Pörtner et al., 2014). Seminal time series studies have documented distribution changes (e.g., Chavez et al., 2003; Edwards et al., 2004; Poloczanska et al., 2013), mismatches in phenologies across the components of the ecosystem (Beaugrand and Reid, 2003) and increased competition leading to a decline of specialist species (Clavel et al., 2010). However few studies assess the potential for evolutionary adaptation in response to climate change in marine species (Dam, 2013; Lohbeck et al., 2012), possibly because the marine ecosystem is often considered to be one of few barriers with limited opportunity for local adaptations (Norris, 2000). Vast population sizes and high dispersal potential in marine plankton are often suggested to limit spatial population structuring. Despite the postulated scarcity of barriers in the ocean, examples of geographic isolation (Casteleyn et al., 2010) and adaptation to local climate exist (Helmuth et al., 2006; Sanford and Kelly, 2011).

The emergence of the Panama Isthmus is an exemplary case of ecosystem reorganisation (Fig. 1). Classically, the focus has been put on the effect of the emerging physical barriers on marine organisms as a potential trigger to speciation (Chaisson, 2003; Knowlton et al., 1993; Miura et al., 2011). However, the closure of the Central American Seaway also led to a reorganisation of ocean circulation that significantly changed the physico-chemical conditions

on both sides of the Isthmus (Haug and Tiedemann, 1998; Haug et al., 2001; Keigwin, 1982; Schmidt, 2007). The exchange between Pacific and northwest Atlantic waters gradually declined between 8 and 5 Ma (Frank et al., 1999), resulting in a shoaling of the Central American Seaway to less than 100 meters by 4.7 Ma and restricting surface water exchange (Haug et al., 2001). By 4.2 Ma, the seaway was generally closed, though Pacific waters episodically breached into the Caribbean across a still-submerged sill or by short-lasting re-openings (Haug et al., 2001). The closure created a habitat separation, both, physically through the Isthmus but also via a separation of the physio-chemical environment (Fig. 1c). Today, surface water temperatures are not much different in both environments (Fig. 1b), but the Caribbean has a much larger mixed layer thickness and higher salinity than the Pacific. The surface waters of the eastern equatorial Pacific are influenced by upwelling of nutrient and CO<sub>2</sub>-rich waters, leading to a shallow nutri- and thermocline and a lower pH (Fig. 1b) (Schmidt, 2007; Zhang et al., 2012).

Planktic foraminifers have an excellent fossil record, which allows us to assess the impact of environmental change and habitat separation. Their morphology has been shown on a geographical scale to reflect environmental preferences (Schmidt et al., 2004a) and genetic diversity (André et al., 2014; e.g., Darling and Wade, 2008; e.g., de Vargas et al., 2001), and temporally to reflect environmental changes (e.g., Malmgren et al., 1983; Norris et al., 1994; Renaud and Schmidt, 2003) and evolution (Malmgren and Kennett, 1981; Norris et al., 1996). We focused on two morphospecies with contrasting ecologies: the deep-dwelling lineage *Globorotalia plesiotumida-tumida*, and the shallow-dwelling *Trilobatus sacculifer*. The deep-dweller should have been affected prior to the surface dweller by the progressive closure of the seaway, as the habitat separation at depth would be expected to predate the surface water separation.

There are a number of competing hypotheses about the response of planktic foraminifers to the physical separation and alteration of their environments: (1) No divergence may occur,

because of world-wide gene flow. (2) Divergence could occur due to the physical separation of populations via the simple break down of gene flow, known as vicariance (Guarnizo et al., 2009). (3) The divergence of the environment itself could select for different adaptation. Here we construct long-term records of planktic foraminifer size and shape and sea surface temperature from either side of the Isthmus to test these hypotheses.

## **2 Materials and Methods**

### *2.1 Background on the investigated species*

*Trilobatus sacculifer* (Brady 1877, Fig. 2 top) is widespread in subtropical and tropical waters (Bé and Tolderlund, 1971). Its stratigraphic range extends from the early Miocene to the present day (Kennett and Srinivasan, 1983). The species' photosynthetic symbionts (Bé et al., 1982) restrict its habitat to the photic zone with a maximum habitat depth at the base of the mixed layer (Bé, 1965; Hecht and Savin, 1972). Its final chamber may take on different shapes: typically it is elongate and sac-like ('sacculifer' (S) morphotype), but it may also be undifferentiated (most frequently referred to as *Gs. or. T trilobus*, herein 'trilobus' (T) morphotype) or occasionally smaller than the previous chamber (kummerform, K). Unexpectedly, recent genetic studies provided evidence that these morphotypes are not linked to genetic differences (André et al., 2013; Aurahs et al., 2011). While the morphological variability has been comprehensively recorded through laboratory experiments (e.g., Bijma et al., 1990a; Bijma et al., 1990b; Hemleben et al., 1989), the biological significance of the sac-like chamber is unknown. In addition to *T. trilobus*, *T. sacculifer* grades with *T. immaturus* and *T. quadrilobatus* the latter being preferentially used for the Miocene morphotypes (André et al., 2013).

The *Globorotalia plesiotumida-tumida* lineage (Fig. 2 bottom) encompasses the evolution from the Late Miocene ancestral morphospecies *Gt. plesiotumida* (Banner and Blow, 1965) to *Gt.*

*tumida* (Brady, 1884) diachronically around the Miocene/Pliocene boundary (Malmgren et al., 1983). A third short-living morphotype in this transition from 6.2 to 5.8 Ma has been described recently (Hull and Norris, 2009). We have deliberately avoided sampling this short range variant to focus on the main transition. Morphological evolution in the lineage is reflected by an increase in size, thicker secondary calcification and changes in convexity and inflation of the test (Malmgren et al., 1983). This asymbiotic, deep-dwelling lineage calcifies close to the bottom of the photic zone in the tropics (Bé and Tolderlund, 1971). Adults live predominantly below 100 m (Bé, 1977), thus reflecting sub-thermocline depth changes (Cannariato and Ravelo, 1997). The specimens were considered to belong to the lineage if their final chamber was noticeably projected and had a distinct (early forms) to heavy (later specimens) keel and a robust test (Fig. 2). There is no indication of cryptic species in the modern morphospecies (André et al., 2014).

## 2.2 Material

*Eastern Equatorial Pacific (Ocean Drilling Program, Leg 202).*– Site 1241 is situated at 5°50.570°N/ 86°26.676°W with a water depth 2027 m (Fig. 1a). The surface ecosystem is characterised by annual sea surface temperatures of 27.4 °C, low salinity (~32.8 psu), and a shallow pycnocline (20-40 m depth) (Boyer et al., 2009). An astronomical age model was used between 6 to 2.5 Ma (Tiedemann et al., 2007), augmented with biostratigraphy from 0 to 2.5 Ma (Flores et al., 2006) and older than 6 Ma (Shipboard Scientific Party, 2003).

*Western Caribbean (ODP, Leg 165).* – Site 1000 is situated at 16°33.223°N/79°52.044°W at a water depth of 916 m. Samples younger than 4.80 Ma were obtained from nearby Site 999 at 12°44.639°N/ 78°44.360°W at a depth of 2828 m (Shipboard Scientific Party, 1997). The surface waters above these sites are characterised by annual average temperatures of 27.8 °C, high salinity (~35.9 psu), and a well-developed mixed layer with a depth of <100 m (Boyer et al., 2009).



All age models (Chaisson and D' Hondt, 2000; Kameo and Bralower, 2000) were adjusted to the 2004 timescale (Lourens et al., 2005) facilitating a direct comparison between the Sites. The measurement of environmental conditions and morphology on the same sample ensures synchronicity of sample comparison and environmental interpretation.

The response of both morphospecies was evaluated using geometric morphometric methods. Ten samples were selected on both sides of the Isthmus, with a time resolution of ~1 Myr. The samples were washed through a 63  $\mu\text{m}$  sieve with deionized water, oven dried at ~50 °C and sieved larger than 150  $\mu\text{m}$ . Splits of the sediment fraction > 150  $\mu\text{m}$  were picked completely to obtain, where possible, at least 30 specimens. A total of 782 *T. sacculifer* and 760 *Gt. tumida* were measured. No *Gt. tumida* was found in the Caribbean sample corresponding to 2.3 Ma and only one *T. sacculifer* in the Pacific sample at 5.3 Ma. Similar to other studies (Hull and Norris, 2009), our data does not allow to assess if this rarity is global or a temporary restriction of the species' biogeographic distribution.

The foraminifers were oriented with the final chamber at the top, and mounted on slides. Two views of the oriented specimens were digitized to describe their three-dimensional morphology: a face view of the umbilical side and primary aperture, and a side view with aperture facing the viewer (Fig. 2). Both species contain sinistrally- and dextrally-coiled forms; therefore, a mirror image was used on left-coiled specimens in order to pool both the right- and left-coiled specimens in the morphometric analyses.

### 2.3 Morphometric analysis

The maximum diameter of each specimen and two-dimensional outlines were extracted using the image-processing software OPTIMAS. Each outline was defined by a set of 64 points sampled at equal curvilinear distance. The starting point of the outline analysis was at the suture between the last and penultimate chambers for *T. sacculifer*, and at the contact between the last chamber and the inner whorl for the face view and the tip of the last chamber

for the side view for *Gt. plesiotumida* and *Gt. tumida*. The variation of the radius (e.g. the distance of each point to the centre of the outline) was expressed as a function of the cumulative distance along the outline. This function was decomposed into a sum of trigonometric functions of decreasing wavelengths (harmonics), each being weighted by two Fourier coefficients (FC) (see de Vargas et al., 2001). The zeroth harmonic, proportional to size maximum diameter of the spiral view (MDsp), was used to standardize all other FCs to remove isometric size effects. Measurement noise has been shown to increase with harmonic rank and exceeds 50% of the signal for coefficients higher than the 12<sup>th</sup> harmonic (de Vargas et al. 2001); coefficients up to the 10<sup>th</sup> harmonic were deemed sufficient for shape analysis.

#### 2.4 Environmental Proxies

A comparison with palaeoenvironmental proxies was used to test for the drivers of any morphological change involved. There are a number of possible ways to reconstruct palaeotemperatures, all of which with their own set of limitations (e.g., Henderson, 2002 and references therein). Ideally the habitat temperatures would be reconstructed using Mg/Ca ratios of the same specimen (Anand and Elderfield, 2003). Reconstruction of temperature by Mg/Ca palaeothermometry, though, rests on the knowledge of sea water Mg/Ca concentrations, and several studies suggest that seawater Mg/Ca ratios have varied over the past several Myrs making the calculation of accurate temperatures challenging (see Medina-Elizalde et al., 2008 for discussion).

C<sub>37</sub> alkenones are produced by coccolithophores living in the top few meters of the water column. They represent sea surface temperatures which moderately overestimate the mixed layer habitat temperature for *T. sacculifer* (Seki et al., 2012). We used the  $U_{37}^{K'}$  data published in Seki et al (2010) and Seki et al (2012) and converted it into temperatures using calibrations defined in Sonzogni et al. (1997).

TEX<sub>86</sub><sup>H</sup> temperatures, which are based on marine Thaumarchaeota, reflect subsurface conditions in the tropics (Seki et al., 2012) similar to the habitat of *Gt. Tumida*. The understanding of their calibration is still strongly evolving. We used published data for Site 1241 (Seki et al., 2012) and, following the same analytical protocol, generated new data for Sites 999 and 1000. In brief, sediments were homogenized and lipids were extracted as described in Seki et al. (2012). Analysis of glycerol dialkyl glycerol tetraethers (GDGTs) for TEX<sub>86</sub><sup>H</sup> was performed according to Schouten et al. (2007). The reproducibility of the TEX<sub>86</sub><sup>H</sup> values is typically 0.01, which is equivalent to 0.3 °C (Schouten et al., 2007). We applied the calibration equation:  $T = 68.4 * \text{TEX}_{86}^H + 38.6$  which was obtained from 255 core tops focussing on the temperature to tropical ocean (Kim et al., 2010), to convert our TEX<sub>86</sub><sup>H</sup> values into temperatures. The calibration error for TEX<sub>86</sub><sup>H</sup> is about 2.5 °C (Kim et al., 2010).

## 2.5 Statistics

Due to the high number of shape variables, the dimensionality of the data was first reduced (Sheets et al., 2006) to assess the data. A principal component analysis (PCA on the variance-covariance matrix of the Fourier Coefficients) was performed on the total data set (FCs of the first ten harmonics in spiral and side views). Axes explaining more than 5% of total variance were retained. The amount of between-group to total variance was calculated using a between-group PCA (Culhane et al., 2002).

Differences between Pacific and Caribbean samples from the same time-interval were investigated using non-parametric Kruskal-Wallis tests for size, and non-parametric multivariate analyses of variance (PERMANOVA) for shape. This analysis compares the distribution of Euclidean distances between observed samples to the ones obtained on 9999 permutations.

A linear model was used to investigate the effects of temperature and location (ocean) on size; and of size, temperature and location on shape. In the case of *T. sacculifer*, the morphotype (K, S, and T) was added as a factor in the model.

Even when standardised by size, FCs may still include size-related, allometric variation. Therefore, residuals of a multivariate regression of FCs upon size (MDsp) were calculated to provide size-free shape variables. Using a similar procedure than on the raw FCs, the size-free FCs residuals were first reduced using a PCA, retaining axes explaining > 5% of variance. The influence of temperature and location (and morphotype for *T. sacculifer*) on these size-free variables was investigated using a linear model.

Multivariate analyses and statistics were performed using the R packages ade4 (Dray and Dufour, 2007 ) and ffmanova (Langsrud and B.-H., 2012) and Past (Hammer et al., 2001).

### **3 Results**

#### *Gt. plesiotumida-tumida*

Size differences between contemporary samples from the two oceans fluctuate through time (Table 2; Fig. 3a), with marked differences during the *Gt. plesiotumida-tumida* transition (7.3 and 6.3 Ma), and in the most recent times slices (1.3 and 0.3 Ma, Table 2). Size is strongly related to temperature, increasing in colder conditions (33% of variance explained, Table 3).

Shape differences between contemporary samples fluctuate through time. They are significantly different in both regions after the closure of the Isthmus (Table 2). A PCA on the raw FCs resulted in 4 axes explaining more than 5% of variance each (35.7%, 17.3%, 11.8%, 6.3%). Between-samples differences explained 23.8% of the total variance. The contribution of size, temperature and location were all significant ( $P < 0.0001$ ; Table 3), with size-related variations being most important (9%).

The importance of size suggests the potential of allometric shape variation. A PCA on size-free FCs residuals resulted in five axes which explained > 5% each (28.9%, 16.5%, 14.0%, 7.5%, 5.5%). Between-group differences explained 20.8% of the total variance. The influence of temperature and location was still significant on this data set ( $P < 0.0001$ ), but temperature is less important than location (temperature: 2.3%, ocean: 3.8% of variance). Size is driven by temperature and therefore allometry could have contributed to the primary role of temperature on raw shape. Consequently, we chose to use size-free axes to visualise shape variations through time (Fig. 3b).

#### *T. sacculifer*

Size differences between contemporary samples were only marginal in *T. sacculifer* (Table 2; Fig. 4a). As in the *plesiotumida-tumida* lineage, size was mostly influenced by temperature (Table 3), explaining 15% of variance.

Shape differences were only significant in the oldest (9.3 Ma) and in the most recent (0.3 Ma) time intervals. A PCA on raw FCs provided two axes > 5%, explaining 49.5% and 31.3% of variance. Between-sample differences explained 15.4% of the total variance. The contribution of size and temperature were highly significant whereas location had only a marginal effect. The size effect is twice as large as the temperature effect on shape variation (size: 10.6%, ocean: 1.1 %, temperature: 5.9 % of variance explained).

A PCA on size-free residuals led to 2 axes > 5% (53.3%, 26.4%), with between-group differences explaining 11.9% of the total variance (Fig. 4b). The influence of location was still minor whereas the influence of temperature was highly significant (ocean: 1.3%, temperature: 5.1%).

Three morphotypes can be identified within *T. sacculifer*. The rarity of the kummerform type precluded any conclusion about its environmental determinants or its shape evolution. The sacculifer and trilobus types roughly correspond to the two modes of the distribution along the

first shape axis (Fig. 4b). The match between the shape modes and the morphotypes is not perfect, however. Especially in the oldest samples, sacculifer and trilobus types have a similar shape, corroborated by the difficulty of separating the morphotypes visibly. The segregation between the morphotypes is more and more pronounced over time; sacculifer types display morphologies departing from the trilobus type with increasing frequency.

The membership to one of the three morphotypes was included in the models but explain only little size variance compared to temperature (ocean: 0.4 %, temperature: 11.2 %, morphotype: 3.5 %; Table 3). The impact on shape, in contrast, was far larger than the effect of temperature (morphotype: 14 %, temperature: 3 %).

The relationship between size, shape, and temperature is shown in Figure 5. For *Gt. tumida*, size varies more or less linearly with temperature (Fig. 5a). Size, shape and the percentage of sacculifer-type specimens are strongly related to temperature (Fig. 5 c, d, e). The relationship between shape and temperature is more complex. Shape co-varies with temperature in the Pacific series of *Gt. tumida*. Caribbean samples, though, deviate frequently from this trend (Fig. 5b), leading to a large variation around the general shape–temperature relationship. Ancestral morphologies of the *Trilobus* three morphotypes were rather similar, sharing compact shapes (Fig 5e,f). The relationship of sacculifer and trilobus types to temperature develops differently, possibly causing the increase in divergence in more recent times. While the shape of the sacculifer morphotype (Fig. 5e) co-varies strongly with temperature, the trilobus morphotype changes very little with varying temperatures (Fig. 5f). Based on our limited sampling, it seems that the kummerform morphotype follows a pattern similar to the sacculifer morphotype. The sacculifer morphotype thus displays the strongest covariation between shape and temperature, even stronger than the one present in *Gt. tumida*.

To investigate this further, we analysed the relationship between shape and environment, separating sacculifer and trilobus morphotypes. As for the whole species, the low variance explained indicates a strong intra-sample variance. In the sacculifer type, the morphologies

differed between the two oceans only in the most recent sample, significantly after the closure of the Isthmus (Table 2). Ocean was never a significant factor, whereas temperature explained 5-9 % of size and shape variance (Table 3). The trilobus morphotype displayed differences between locations in the oldest time slice. It is important to note that we lack confidence in our assignment of morphotypes in this time-slice as overall the morphologies are very similar (Table 2). Size is strongly related to temperature (24 % of variance explained). Shape was slightly related to temperature (2-3%), whereas the effect of location was not significant.

## **4 Discussion**

The closure of the Central American seaway represents the first time in the evolutionary history of modern marine plankton that the connection across *all* tropical seaways was interrupted. As a result species had to move across a wide range of habitats to exchange genetic information between ocean basins. The general expectation in response is that the progressive shoaling of the Central American seaway and the formation of the Isthmus of Panama would (1) trigger differentiation due to isolation and (2) impact deep-dwelling species first and hence affect *Gt. tumida* prior to *T. sacculifer*.

### *4.1 Closure of the Isthmus as a trigger of differentiation: a validation*

Despite known world-wide genetic exchanges in foraminifers (e.g. Darling et al., 2000), both morphospecies displayed a differentiation in shape of Atlantic and Pacific populations after the closure of the seaway. This differentiation occurred significantly earlier for the deep-dwelling *Gt. tumida* than for the shallow-dwelling *T. sacculifer*. This differentiation could be due to vicariance but a local adaptation to progressively differing environments can also cause this differentiation as the Isthmus of Panama generated a split in the environmental conditions in the eastern equatorial Pacific and the Caribbean (Schmidt, 2007).

### *4.2. Size trends: A physiology-driven response to temperature?*

Adult size in planktic foraminifers is the outcome of environmentally controlled growth rates (see Schmidt et al., 2006 and references therein). Therefore, physiology-driven size response is a good candidate to explain the observed trends. Large size in this group indicates optimal environmental conditions (Hecht, 1976; Schmidt et al., 2004a; Schmidt et al., 2004b). Assuming that the temperature effect on physiology and size is stable over time allows a cautious comparison to the modern temperature niche. The modern SST optimum for *T. sacculifer* is 27 °C (Lombard et al., 2009; Schmidt et al., 2004a), slightly colder than the majority of our record. The cooling temperatures over the studied interval therefore move temperatures towards more optimal conditions and allow growth to larger size (Fig. 3 and 5). This interpretation is based on the assumption that the temperature optima have not change significantly over time. The optimal habitat temperatures for *Gt. tumida* today are between 12°C and 17 °C (estimated by maximum abundance of this species and temperature at 200 m (Prell et al., 1999)). The increase in size with cooling similarly suggests temperatures moving closer to the specific optimum. Hence, physiology-driven response to temperature is sufficient to explain the size variation of both morphospecies over time and the relationship does not change over time.

#### 4.3. Shape changes: temperature and vicariance

Our temperature-driven changes in size affect shape due to allometric variations. These allometric variations mask subtle changes in shape departing from the allometric relationship (Girard and Renaud, 2008). The effect of size as a major driver of shape evolution is well known (Marroig and Cheverud, 2005). We assessed size-free shape variations to investigate how they may relate to environmental changes beyond the signal driven by the size-temperature relationship. The similarity of our results on raw and size-free data suggests that shape response was not merely a result of allometry.

A differentiation of Pacific and Atlantic stocks in the shallow-dweller *T. sacculifer* occurred several millions years after the closure of the Isthmus, concomitant with divergence in the



temperature conditions at both locations. Understanding the response of *T. sacculifer* is complicated by the occurrence of several morphotypes. This species is known for both its phenotypic variability but also its global genetic homogeneity (André et al., 2013). The driver of the plasticity is still poorly understood and experiments result in a wide range of responses. Bé et al. (1981) found that increased feeding frequency increases sac-like chamber formation and growth rate, resulting in larger final test sizes in these experiments. Bijma et al. (1992) in experiments at the same location found an interplay between feeding and light influencing the formation of the sac-like chamber. While variable, they found that lower feeding rates resulted in a greater number of sac-like phenotypes. High light intensity, typically associated with oligotrophic environments such as the Caribbean, lead to higher frequency of sac-like chambers (Bijma et al., 1992; Caron et al., 1981). Both observations might explain the high abundance of *sacculifer* morpho types in the oligotrophic Caribbean compared to the Pacific. Bijma et al. (1992) found that low light, representing deeper waters, results in a higher number of kummerform types in their experiments but the low abundance in our samples hinders us from drawing inferences.

Whatever the process, mean shape of *T. sacculifer* and the shape of each morphotype appears to be responding to temperature over time. The relative abundance of both dominant morphotypes is correlated to temperature. The controlling mechanism may not be temperature *per se* but could be a knock-on effect of temperature-related shifts in oceanographic conditions (e.g. the nutrient supply to prey species). This raises further questions about the determinism of the morphotypes, but clearly shows that the response of *T. sacculifer* was not driven by vicariance but by the progressive differentiation of the environmental conditions on both sides of the Isthmus. Changes in shape influence buoyancy (Caromel et al., 2014). The more elongated Atlantic type and its symbionts would therefore be able to stay longer in the surface water compared to the more rounded Pacific type. The change in shape would also allow the morphotype to grow larger without losing relative internal volume and hence reproductive success (Caromel et al., 2014). This morphological change therefore might facilitate the

exploitation of the mixed layer habitat, benefiting the symbionts and facilitating growth to large sizes. This ecological advantage is highlighted by the globally highest abundances (>40% of the assemblage) of *T. sacculifer* in the tropical Atlantic Ocean (Prell et al., 1999).

Regarding the deep-dweller *Gt. tumida*, temperature also appears as a significant driver of shape change. Differences between the two oceans, which cannot be linked to temperature differences, appear significant as well. We therefore suggest that vicariance played a role in the differentiation of Caribbean and Pacific populations of this species.

Physiology-driven changes may contribute to this reaction to the environment, but such processes are most probably reinforced by sorting of genetic variants from a vast standing stock. Overall our study highlights the high ability of foraminifers to respond to environmental changes, mostly following temperature (or temperature-related) variations, an environmental parameter which we expect to change rapidly over the coming decades. While phenotypic plasticity, as strongly expressed in *T. sacculifer*, is generally thought to shield genetic variation from selection (Senner et al., 2015), it may also facilitate adaptation by allowing survival in new environments in the first place (Badyaev, 2005; Ghalambor et al., 2007; Price and Qvarnström, 2003).

#### 4.4 Conclusions

Our main finding is that the size and shape evolution of both investigated species is strongly influenced by temperature, despite their different ecology. This raises concerns about the impacts of current climate change on foraminifers. This can be directly on the organisms by impacting on growth and volume which relates to the numbers of gametes produced. Indirectly, changes to the amount carbonate produced by each specimen impacts the production of carbonate in the ocean and thereby global biogeochemical cycles. The open question therefore is if these species, and potentially all planktic foraminifers, will be able to track their habitat given that environmental changes are currently occurring at a rate outside

foraminifers' evolutionary experience. A physiological response and the selection of an available phenotype or genotype from a large standing stock would both facilitate a rapid response to environmental changes.

#### **Author contributions**

DNS conceived the idea, AC collected the morphometric data, JR and OS the temperature data. SR performed the morphometric analysis. DNS and SR interpreted the data. AC, DNS, SR wrote the paper. All authors contributed to the manuscript and gave final approval for publication. We have no competing interests.

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673 **Tables**

674 Table 1. Sample size and summary of the data. N: sample size for morphometrics (for *T.*  
 675 *sacculifer* also divided by morphotypes S(accumifer), T(rilobus) and K(ummeform)).  
 676 TEXtemp: thermocline temperature [°C]; UKtemp: sea surface temperature [°C]. MDsp:  
 677 mean size of the foraminifer (maximum diameter in spiral view) [μm].

<i>Gt. tumida</i>					<i>T. sacculifer</i>					
Area	Age	N	TEXtemp	MDsp	N	NK	NT	NS	UKtemp	MDsp
	[Ma]		[°C]	[μm]					[°C]	[μm]
CAR	0.3	43	24.3	766	39	1	37	1	24.6	562
CAR	1.3	86	23.7	810	64	3	47	14	28.3	465
CAR	2.3	0	23.2		67	0	42	25	28.2	521
CAR	3.3	32	24.2	678	32	0	20	12	28.5	438
CAR	4.3	28	23.7	543	45	1	21	23	29.4	413
CAR	5.3	100	25.3	619	37	0	14	23	30.4	434
CAR	6.3	30	26.1	370	37	0	17	20	30.4	417
CAR	7.3	86	26.3	385	65	0	27	18	30.4	387
CAR	8.3	57	26.9	365	35	0	16	19	30.4	436
CAR	9.3	5	26.5	344	28	0	8	20	30.4	360
PAC	0.3	29	22.1	633	31	0	20	11	27.4	603
PAC	1.3	51	24.1	628	47	1	33	13	28.1	534
PAC	2.3	37	23.3	685	60	1	37	22	28.6	477
PAC	3.3	34	24.0	790	34	0	20	14	28.8	486
PAC	4.3	40	24.9	651	30	0	14	16	28.9	469
PAC	5.3	34	24.1	656	1	0	0	1	29.0	546
PAC	6.3	32	25.3	478	33	1	13	19	28.0	471

PAC	7.3	13	26.9	516	31	0	15	16	29.0	389
PAC	8.3	21	25.6	354	38	1	12	25	29.5	409
PAC	9.3	2	26.0	499	28	0	9	19	29.5	394

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Table 2. Size and shape differences between contemporary samples from the Caribbean and the Pacific Ocean. Size differences were tested on the maximum diameter in spiral view (MDsp) using non-parametric Kruskal-Wallis tests. Shape differences were tested on the set of principal axes explaining > 5% of variance, on the raw and size-free (SF) data, using a non-parametric multivariate analyses of variance (PERMANOVA). Probabilities of the tests are given. In bold:  $P < 0.001$ ; in italics:  $P < 0.05$ . *Gt. tumida*: Note that the latest pair includes 7 specimens only. *T. sacculifer*: results are provided for the whole assemblage, and for S and T morphotypes separately.

	<i>Gt. tumida</i>			<i>T. sacculifer</i>			S type			T type		
	MDsp	Raw	SF	MDsp	Raw	SF	MDsp	Raw	SF	MDsp	Raw	SF
0.	<b>0.000</b>	<b>0.000</b>	<b>0.000</b>	0.1228	<b>0.000</b>	<b>0.000</b>	0.262	<b>0.000</b>	<b>0.000</b>		0.085	0.080
3	<b>6</b>	<b>1</b>	<b>1</b>		<b>1</b>	<b>1</b>	6	<b>2</b>	<b>1</b>		4	5
1.	<b>&lt;</b>	<b>0.000</b>	<b>0.000</b>	<i>0.0053</i>	0.079	0.187	<i>0.017</i>	0.136	0.341	0.120	0.866	0.623
3	<b>0.000</b>	<b>3</b>	<b>1</b>		7	9	3	5	3	5	4	1
2.				<i>0.0406</i>	0.690	0.491	0.054	0.683	0.314	0.717	0.288	0.506
3					8	6	2	2	3	0	9	8
3.	<i>0.017</i>	<b>0.000</b>	<b>0.000</b>	0.0543	0.165	0.538	0.074	0.774	0.964	0.354	0.000	0.001
3	6	<b>1</b>	<b>1</b>		4	6	2	5	9	5	7	0
4.	<i>0.008</i>	<i>0.001</i>	<b>0.000</b>	<i>0.0083</i>	0.609	0.919	0.106	0.421	0.580	<i>0.027</i>	0.108	0.099
3	6	0	<b>1</b>		3	4	0	5	3	9	5	9
5.	0.201	<b>0.000</b>	<b>0.000</b>		0.386	0.182					0.286	0.166
3	2	<b>1</b>	<b>1</b>		0	8					1	4
6.	<b>0.000</b>	<i>0.001</i>	<i>0.001</i>	<i>0.0146</i>	0.177	0.133	0.414	0.149	0.135	<i>0.002</i>	0.101	0.515
3	<b>1</b>	5	8		1	4	4	3	8	4	6	4
7.	<b>0.000</b>	<i>0.005</i>	0.064	0.8111	0.577	0.600	0.212	0.295	0.479	0.166	<i>0.003</i>	<i>0.016</i>
3	<b>1</b>	5	4		2	9	4	9	6	7	7	3
8.	0.420	<i>0.001</i>	<b>0.000</b>	0.1709	<i>0.020</i>	0.062	0.265	0.286	0.331	0.413	0.099	0.153
3	6	8	<b>1</b>		4	4	2	6	3	7	1	7
9.	<i>0.052</i>	0.808	0.474	0.1119	<b>0.000</b>	<b>0.000</b>	0.289	<i>0.006</i>	<i>0.018</i>	0.325	<b>0.000</b>	<b>0.000</b>
3	8	9	6		<b>3</b>	<b>5</b>	8	0	1	4	<b>2</b>	<b>5</b>

Table 3. Percentage of variance and probability of the different factors (size, ocean, temperature, and morphotype, depending on the model) explaining size and shape for *Gt. tumida* and *T. sacculifer*. In bold effects with  $P < 0.0001$ .

		% variance				P			
		Size	Ocean	Temperature	Types	Size	Ocean	Temperature	Types
<i>Gt. tumida</i>	Size		1.2	<b>33.2</b>			0.0003	<b>&lt;0.0001</b>	
	Shape, raw	<b>8.6</b>	<b>1.8</b>	<b>3.9</b>		<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	
	Shape, sizefree		<b>3.8</b>	<b>2.5</b>			<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	
<i>T. sacculifer</i>	Size		<b>0.2</b>	<b>16.1</b>			0.128	<b>&lt;0.0001</b>	
	Shape, raw	<b>10.6</b>	1.1	<b>5.9</b>		<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	
	Shape, sizefree		1.3	<b>5.1</b>			<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	
<i>With types</i>	Size		<b>0.4</b>	<b>11.2</b>	<b>3.5</b>		0.047	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>
	Shape, raw	<b>9.0</b>	0.8	<b>1.8</b>	<b>14.0</b>	<b>&lt;0.0001</b>	0.0004	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>
	Shape, sizefree		1.0	<b>1.8</b>	<b>14.0</b>		0.0001	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>
<i>sacculifer type</i>	Size		0.4	<b>8.5</b>			0.193	<b>&lt;0.0001</b>	
	Shape, raw	<b>8.7</b>	0.6	<b>6.9</b>		<b>&lt;0.0001</b>	0.106	<b>&lt;0.0001</b>	
	Shape, sizefree		0.7	<b>5.3</b>			0.0823	<b>&lt;0.0001</b>	
<i>trilobus type</i>	Size		0.2	<b>23.5</b>			0.309	<b>&lt;0.0001</b>	
	Shape, raw	<b>8.2</b>	0.0	0.9		<b>&lt;0.0001</b>	0.8492	0.0145	
	Shape, sizefree		0.0	<b>2.3</b>			0.946	<b>&lt;0.0001</b>	

## Figures

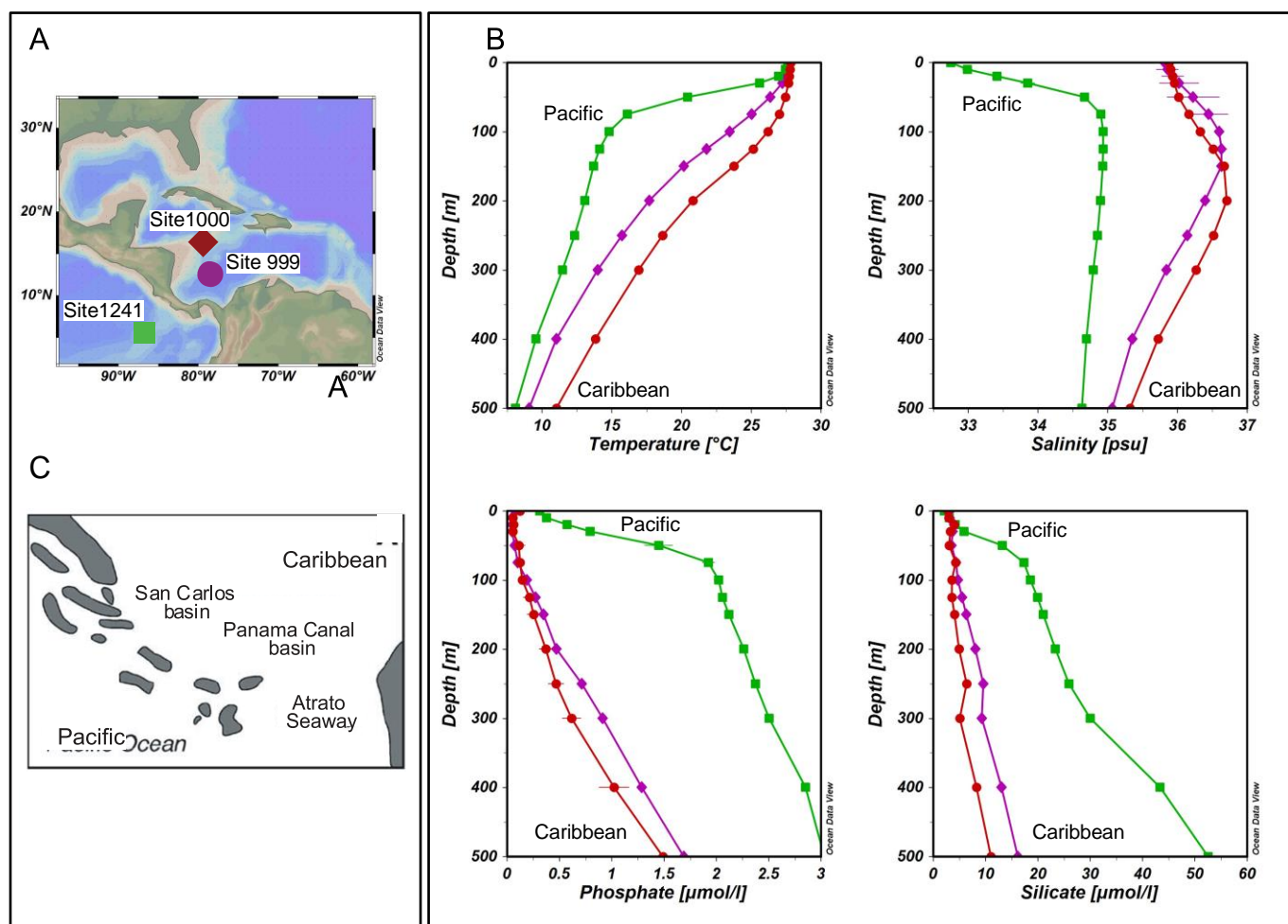
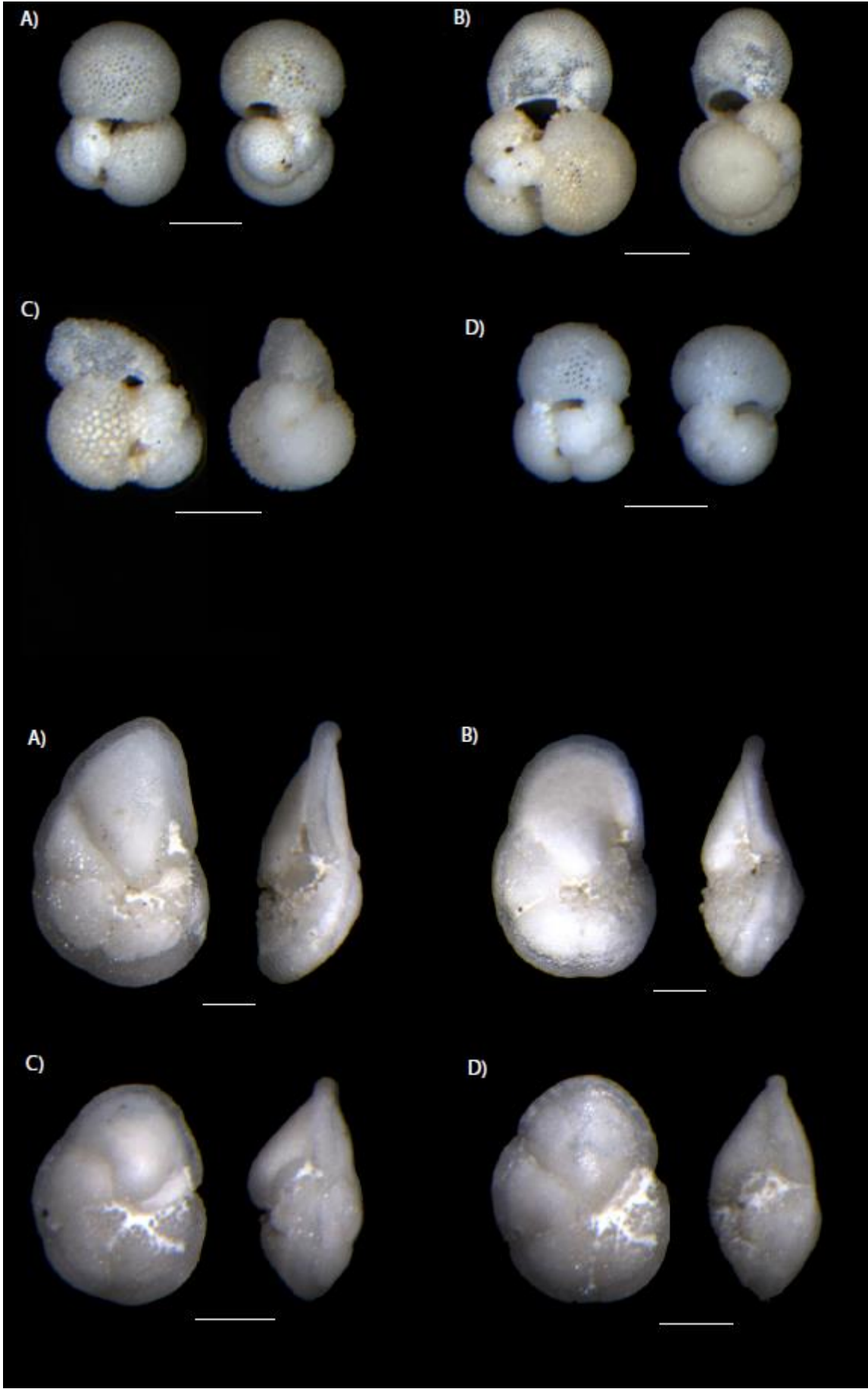


Figure 1: Overview of the core locations and their environmental conditions. (A) core locations and (B) environmental characteristics: temperature, salinity, phosphate and silicate. Note the strong modern differences in water column structure and nutrient distribution between the two regions. Green circles – Pacific Site 1241, Red squares –Caribbean Site 1000, purple diamond – Caribbean Site 999; same colour coding is used through the figures. The map was generated with Ocean Data View (Schlitzer, 2006). The environmental data is from World Ocean Atlas (Garcia et al., 2006).



709 Figure 2: Overview of the morphological variability within the selected species. TOP *T.*  
710 *sacculifer*: A, D typical ‘trilobus’ morphotypes without sac-like final chamber (A 202-1241-2H1,  
711 D 202-1241-25H6); B, C morphotypes with inflated (B) and typical (C) sac-like final chamber  
712 (B 202-1241-2H1, C 165-999-2H2); all specimens in spiral view and side view showing the  
713 primary aperture. BOTTOM *Gt. tumida* – *plesiotumida* lineage: A, B *Gt. tumida* with different  
714 final chamber shapes (202-1241-9H2) and C, D *Gt. plesiotumida* with different degrees of  
715 inflation (202-1241-18H7); all specimens in umbilical view and side view showing the primary  
716 aperture. Scale bars: 200  $\mu\text{m}$ .

717

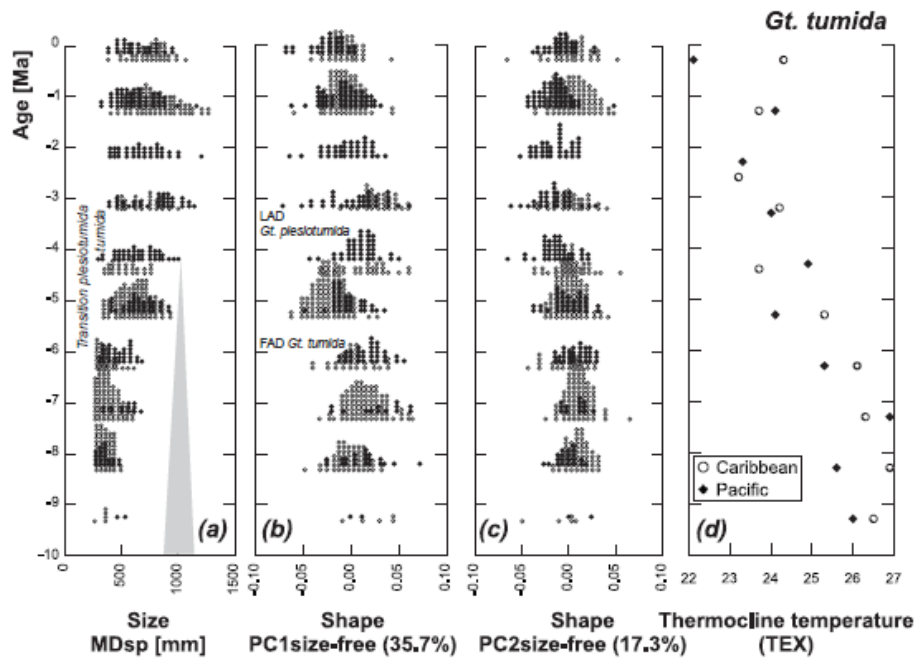


Figure 3. Temporal variations in size and shape of *Gt. tumida* and the associated temperature changes. (a) Size [ $\mu\text{m}$ ], estimated by maximal diameter in spiral view; (b, c) Shape variations, estimated by the first (b) and second (c) axes of PCA on the Fourier coefficients, using both sides combined. In a, b and c, each dot corresponds to an individual specimen. (d)  $\text{TEX}_{86}^{\text{H}}$  [ $^{\circ}\text{C}$ ] derived thermocline temperatures; large symbols correspond to the value used for comparison with morphometrics. The noticeable increase in size between 6.3 and 5.3 Ma is a response of the transition from *Gt. plesiotumida* to *Gt. tumida*. The divergence in shape of the Pacific and Atlantic stock between 5.4 and 4.3 is concomitant with the environmental separation of the seaway at the habitat depth of the species which started in the Miocene (indicated by grey wedge).



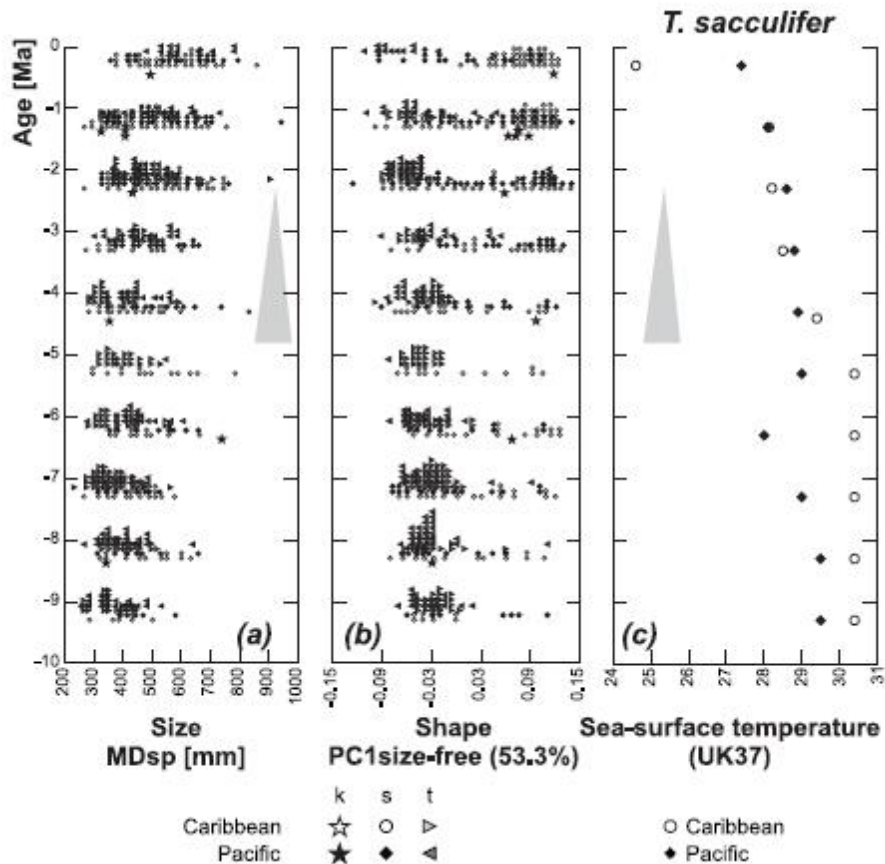


Figure 4. Temporal variations in size and shape of *T. sacculifer* and the associated temperature changes. (a) Size [μm], estimated by maximal diameter in spiral view; (b, c) Shape variations, estimated by the first (b) and second (c) axes of a PCA of the size-free Fourier coefficients, using both sides combined. In a, b and c, each dot corresponds to an individual specimen. (d)  $U_{37}^K$  [°C] derived sea-surface temperatures (Seki et al., 2010, 2012). The progressive limitation of surface water exchange and the final closure are indicated by the grey wedge. Note the size increase in *T. sacculifer* and the significant among sample variations. Combined with a gradual change in shape, this change appears to be independent of the emergence of the Isthmus. Top panel for each of the dot plots Pacific and bottom panel Caribbean.

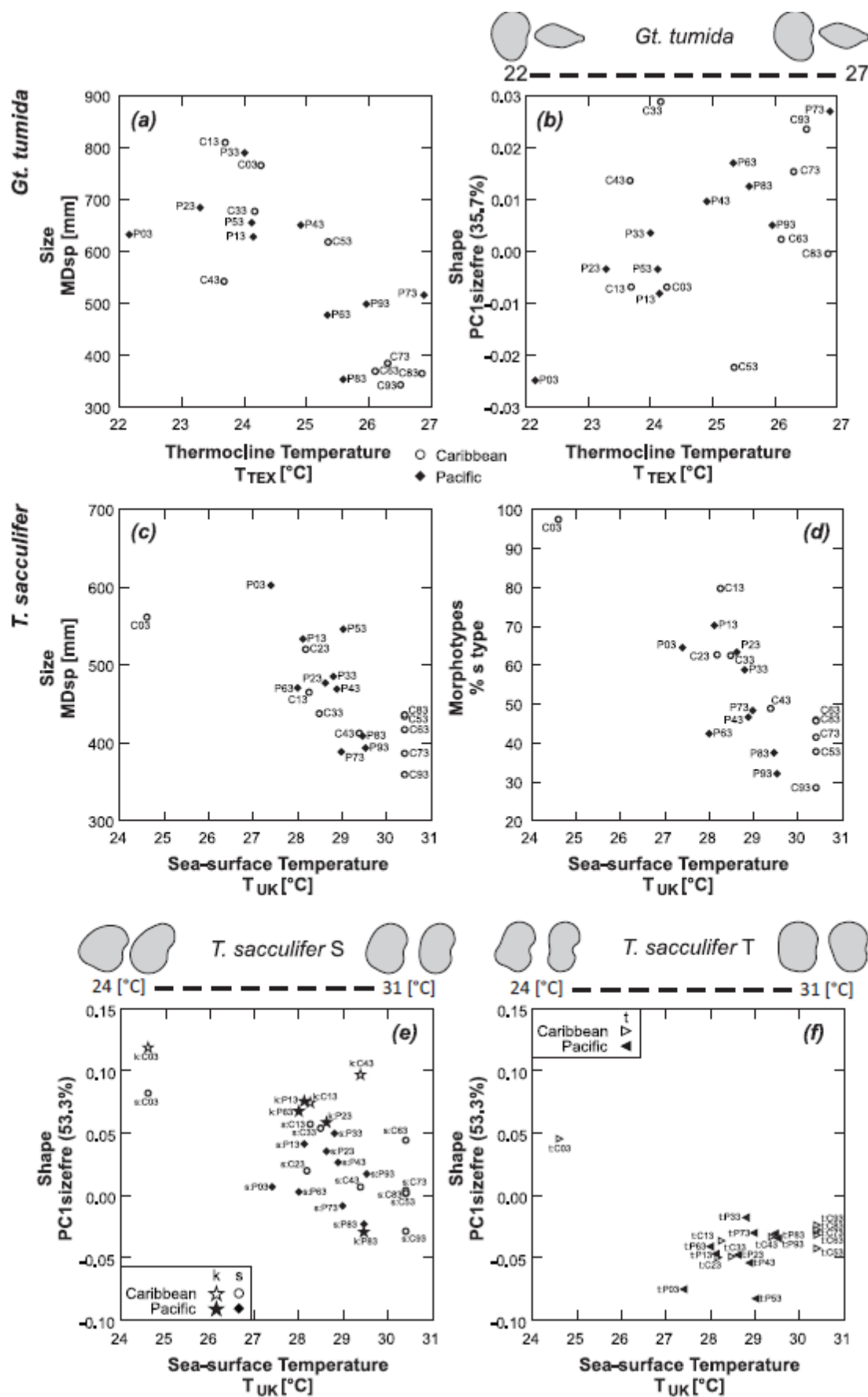


Figure 5. Relationship between mean morphological parameters and temperature estimates.

(a ,b) *Gt. tumida* vs.  $\text{TEX}_{86}^{\text{H}}$  [°C] derived temperature estimates = thermocline temperatures.

(c, d, e, f) *T. sacculifer* vs.  $\text{U}_{37}^{\text{K}}$  [°C] derived temperature estimates = sea surface temperatures.

(a, c) Size [ $\mu\text{m}$ ] (maximal diameter in spiral view) of *Gt. tumida*. (b) *Gt. tumida* shape (first axis of a PCA on size-free Fourier coefficients, both sides combined) (c) Size [ $\mu\text{m}$ ] of *T. sacculifer*, all morphotypes averaged by sample. (d) Percentage of morphotypes of *T. sacculifer*. (e) Shape of the sacculifer and kummerform morphotypes of *T. sacculifer*. (f) Shape of the trilobus morphotype of *T. sacculifer* (note that PC axes are the same on panels e and f, since the PCA was done on all *T. sacculifer*). For plots of shape vs. temperature, a visualisation of the shape changes vs. temperature is provided above the corresponding plot (b: *Gt. tumida*; e: S(acculifer) and f: T(rilobus) morphotypes of *T. sacculifer*). The visualisation was obtained using a multivariate regression of the raw FCs vs. temperature.